ON THE INHERITANCE OF COLOR IN A FRESH-WATER FISH, *APLOCHEILUS LATIPES* TEMMICK AND SCHLEGEL, WITH SPECIAL REFERENCE TO SEX-LINKED INHERITANCE

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TABLE OF CONTENTS

INTRODUCTION

Aplocheilus latipes (Japanese name 'Medaka') is a small fish of the family Poeciliidæ very common in our streams and paddy fields. Its colored varieties are well known as ornamental fishes. Since, on account of their small size, a great many individuals may be bred within a small space, they are very suitable objects for breeding experiments. Since

1913, experiments have been carried on to investigate the genetic behavior of their body color, and some interesting results have been obtained so that I think it will be worth while to report them here as a contribution to knowledge about the genetics of fishes which has till now been very rarely investigated.

MATERIALS AND METHODS

The brown-black variety was obtained wild from nature, while all others were bought from a fancier. Among them, I was able to distinguish four varieties, viz., orange-red, white, orange-red variegated with black, and white variegated with black (plate 1, figures 1, 3, 5, 2).

In nature this fish seems to live two years. The young, hatched out in summer, spawn at the same season of the next year and soon die, so that in autumn all fishes in nature are young and small. In culture their life may be prolonged one year more.

When the fishes are well nourished, they spawn every day early in the morning, from the beginning of May to the end of August. According to my observations, the number of eggs deposited daily averages 25; the maximum was 71. The female carries the spawned egg-mass about half a day, when not disturbed.

For the purpose of rearing the young, I stripped off the egg mass from the female with the fingers, and then put the egg masses in a porcelain vessel about one foot in diameter, one vessel containing not more than five egg masses. The young hatch in about one week after spawning in the hottest part of our summer.

As food for young just hatched, either powder of heated wheat grains or any dried flesh is good, while fish which are somewhat grown, if nourished with small earthworms (Tubificidæ), grow quite rapidly. The body of a full-grown fish measures on the average 30 mm in length, without the tail-fin. An aquarium of four square feet in surface area and measuring one foot and a half in depth may contain 200 adult fishes in a healthy condition, if proper care is taken to change the water.

EXPERIMENTAL RESULTS

For the sake of brevity, varieties which are more accurately described as brown-black, blue-black, orange-red, orange-red variegated with black, and white variegated with black, will hereafter be designated simply as brown, blue, red, red variegated, and white variegated, respectively. The number within parentheses denotes the year when the mating was made.

EXPLANATION OF PLATE

Originally drawn to twice natural size, but reduced to one and three-fourths times natural size in reproduction.

Figure 1.—Orange-red.
Figure 2.—White variegated with black.
Figure 3.—White.
Figure 4.—Brown-black, wild form.
Figure 5.—Orange-red variegated with black
Figure 6.—Blue-black.

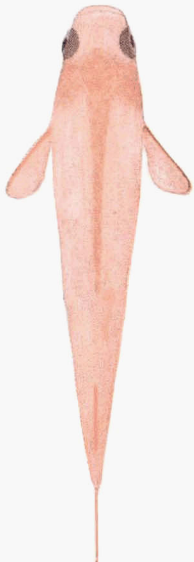
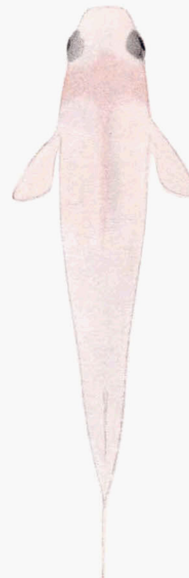


Fig. 1


Fig. 2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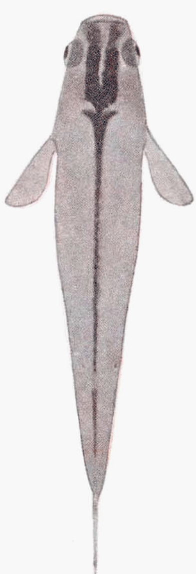
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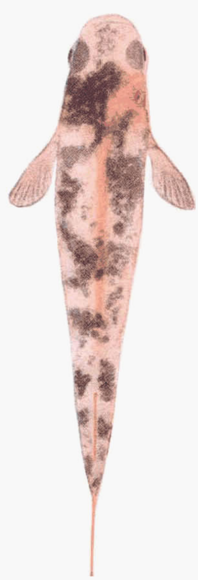
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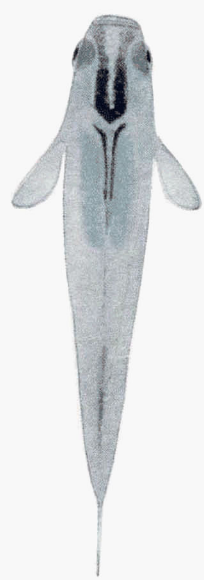
Fig. 6

Crosses between brown and red

Experiment 1

Brown males were mated to red females (1913). The F_1 progeny were brown, and indistinguishable from the wild form. The result of their inbreeding (1914) was as follows:

	Brown	*Red*
Observed	865	292
Expectation	*867.75*	*289.25*

Experiment 2

Back-cross of experiment 1. Heterozygous brown females were mated to pure red males (1914). The result was

	Brown	*Red*
Observed	937	924
Expectation	930.5	930.5

Crosses between brown and variegated red

Variegated red individuals bought from a fancier were all heterozygous for variegation, and produced red individuals when bred *inter se*. By selective mating, I obtained in 1915 homozygous variegated red individuals with which to perform further experiments.

Experiment 3

Homozygous variegated red males were mated to pure brown females (1915). The offspring were indistinguishable from individuals of the pure-bred brown strain. Their inbreeding (1916) gave the following result:

	Brown	*Variegated red*
Observed	229	77
Expectation	*229.5*	*76.5*

Experiment 4

Back-cross of experiment 3. Heterozygous brown males were bred to homozygous variegated red females (1916). The result was

	Brown	*Variegated red*
Observed	148	148
Expectation	*148*	*148*

Crosses between variegated red and red

Experiment 5

Homozygous variegated red females were mated to homozygous red males (1915). The F_1 offspring were all variegated red similar to the variegated parents. The F_2 offspring (1916) were as follows:

	Variegated red	Red
Observed	457	161
Expectation	*463.5*	*154.5*

Experiment 6

Back-cross of experiment 5. Heterozygous variegated red males were bred to homozygous red females (1916). The result was

	Variegated red	Red
Observed	216	200
Expectation	*208*	*208*

Though crosses reciprocal to the above six breeding experiments were carried out, their details are omitted here because they have given exactly similar results.

Crosses between white females and red males

White color is a sex-linked character, as explained below, and so the sex of the progeny was carefully examined in all the following experiments. To avoid error the examination as to sex was made after the fishes were fully grown, females being distinguished as such by spawning and males by having produced the long dorsal and anal fins.

Experiment 7

White females were mated to homozygous red males (1914). The F_1 offspring were all red. The F_2 offspring (1915) were as follows:

	Red	White
Observed	217	76
Expectation	*219.75*	*73.25*

The result of sex examination was

	Red		White	
	♀	♂	♀	♂
Observed	41	76	43	0
Expectation	*39*	*78*	*43*	*0*

Experiment 8

Back-cross of the above experiment. Heterozygous red males were bred to white females (1915). The result was

	Red	White
Observed	519	502
Expectation	*510.5*	*510.5*

The result of sex examination was

	Red		White	
	♀	♂	♀	♂
Observed	2	251	197	1
Expectation	*0*	*253*	*198*	*0*

Crosses between white females and variegated red males

Experiment 9

Pure variegated red males were bred to white females (1917). The F_1 offspring were all variegated red. The F_2 offspring (1918) were

	Variegated red	*Variegated white*	*Red*	*White*
Observed	279	95	96	30
Expectation	*281.25*	*93.75*	*93.75*	*31.25*

The result of sex examination was

	Variegated red		*Variegated white*		*Red*		*White*	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	46	90	59	0	17	37	17	0
Expectation	*45.3*	*90.6*	*59*	*0*	*18*	*36*	*17*	*0*

Crosses between white females and brown males

Experiment 10 A

Homozygous brown males were mated to white females (1915). The F_1 offspring were all brown. The F_2 offspring (1916) were

	Brown	*Blue*	*Red*	*White*
Observed	248	57	53	21
Expectation	*213.12*	*71.04*	*71.04*	*23.68*

The result of sex examination was

	Brown		*Blue*		*Red*		*White*	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	77	147	56	0	9	37	19	0
Expectation	*74.7*	*149.4*	*56*	*0*	*15.3*	*30.6*	*19*	*0*

Experiment 11 A

Back-cross of experiment 10 A. Heterozygous brown males were mated to white females (1916). The offspring were

	Brown	*Blue*	*Red*	*White*
Observed	387	317	253	246
Expectation	*300.75*	*300.75*	*300.75*	*300.75*

The result of sex examination was

	Brown		*Blue*		*Red*		*White*	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	0	176	214	1	0	135	146	0
Expectation	*0*	*176*	*215*	*0*	*0*	*135*	*146*	*0*

In the last two experiments the deviation of the actual numbers from expectation is rather large, so that I have repeated both experiments.

In order to reduce as much as possible the influence of differential mortality among the young of various colors, records were taken at as early a stage as was possible. First, at a very young stage, i.e., two or three days after hatching, black and colorless young were distinguishable from each other and were separated. Secondly, when the young fish had grown to about 1 cm in length and the development of xanthophore had become conspicuous, those previously classed as "black" were distinguishable either as brown or as blue and were separated accordingly, while those previously classed as "colorless" were distinguishable as red or as white, and were so classified.

Experiment 10 B

Repetition (1917–1918) of experiment 10 A. The first inspection of the F_2 generation gave

	Black	*Colorless*
Observed	2016	667
Expectation	*2012.25*	*670.75*

The black grew into

	Brown	*Blue*
Observed	779	244
Expectation	767.25	*255.75*

The colorless grew into

	Red	*White*
Observed	329	113
Expectation	*331.5*	*110.5*

The result of sex examination was

	Brown		*Blue*		*Red*		*White*	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	71	164	59	0	48	131	62	0
Expectation	*78.3*	*156.6*	*59*	*0*	*59.7*	*119.4*	*62*	*0*

Experiment 11 B

Repetition (1918) of experiment 11 A. The first inspection gave

	Black	*Colorless*
Observed	823	776
Expectation	*799.5*	*799.5*

The black grew into

	Brown	*Blue*
Observed	228	230
Expectation	229	*229*

The colorless grew into

	Red	White
Observed	237	222
Expectation	*229.5*	*229.5*

The result of sex examination was

	Brown		Blue		Red		White	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	2	120	114	0	0	71	62	0
Expectation	*0*	*122*	*114*	*0*	*0*	*71*	*62*	*0*

Crosses between blue and red

Experiment 12

Blue offspring of experiment 10 A were bred to homozygous red males (1917). The progeny were

	Brown	Red
Observed	222	234
Expectation	*228*	*228*

The result of sex examination was

	Brown		Red	
	♀	♂	♀	♂
Observed	62	41	69	48
Expectation	*51.5*	*51.5*	*58.5*	*58.5*

Mating white with white

Experiment 13

The solitary male which was found among the white offspring in experiment 8 is the progenitor of all white males met with in further experiments. He was mated to a white female (1916); the offspring, 149 in number, were all white; 108 of them have grown to maturity, 66 being female and 42 male.

Crosses between blue females and white males

Experiment 14

The object of this experiment was not only to study the genetic constitution of blue individuals, but also to obtain blue males. Blue females, derived from experiment 10 A, were mated to white males (1917). The result was

	Blue	White
Observed	146	151
Expectation	*149*	*149*

The result of sex examination was

	Blue ♀	Blue ♂	White ♀	White ♂
Observed	33	48	51	49
Expectation	*40.5*	*40.5*	*50*	*50*

Crosses between blue and blue

Experiment 15

Blue descendants of experiment 14 were bred *inter se* (1918). The progeny were

	Blue	*White*
Observed	618	200
Expectation	*613.5*	*204.5*

Crosses between brown and blue

Experiment 16

Pure blue females were mated to brown males (1919). The F_1 offspring were all brown. In the F_2 generation (1920), there were

	Brown	*Blue*
Observed	174	59
Expectation	*175*	*58*

Crosses between brown and white

Experiment 17

White males were bred to pure brown females (1917). The F_1 offspring were all brown. The F_2 offspring (1918) were at an early stage

	Black	*Colorless*
Observed	1481	532
Expectation	*1509.75*	*503.25*

The black grew into

	Brown	*Blue*
Observed	477	150
Expectation	*470*	*157*

The colorless grew into

	Red	*White*
Observed	208	69
Expectation	*207.75*	*69.25*

The result of sex examination was

	Brown ♀	Brown ♂	Blue ♀	Blue ♂	Red ♀	Red ♂	White ♀	White ♂
Observed	173	88	0	79	78	34	0	31
Expectation	*174*	*87*	*0*	*79*	*74.6*	*37.3*	*0*	*31*

Experiment 18

Heterozygous brown males which were the F_1 offspring derived from experiment 17, were bred to white females (1918). The offspring were at an early stage

	Black	*Colorless*
Observed	917	988
Expectation	*952.5*	*952.5*

The black grew into

	Brown	*Blue*
Observed	354	339
Expectation	*346.5*	*346.5*

The colorless grew into

	Red	*White*
Observed	363	378
Expectation	*370.5*	*370.5*

The result of sex examination was

	Brown		*Blue*		*Red*		*White*	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	157	0	0	148	165	0	0	126
Expectation	*157*	*0*	*0*	*148*	*165*	*0*	*0*	*126*

Crosses between red and white

Experiment 19

White males were bred to homozygous red females (1917). The F_1 offspring were all red. In F_2 (1918) there were

	Red	*White*
Observed	766	264
Expectation	*772.5*	*257.5*

The result of sex examination was

	Red		*White*	
	♀	♂	♀	♂
Observed	87	42	0	33
Expectation	*86*	*43*	*0*	*33*

Experiment 20

Heterozygous red males produced as F_1 offspring in experiment 19 were bred to white females (1918). The offspring were

	Red	*White*
Observed	330	324
Expectation	*327*	*327*

The result of sex examination was

	Red		*White*	
	♀	♂	♀	♂
Observed	135	0	0	153
Expectation	*135*	*0*	*0*	*153*

Crosses between variegated red females and white males

Experiment 21

White males were mated to homozygous red variegated females (1917). The F_1 offspring were all red variegated. In the F_2 generation (1918), there were

	Variegated red	*Variegated white*	*Red*	*White*
Observed	157	50	49	18
Expectation	*154.125*	*51.375*	*51.375*	*17.125*

The result of sex examination was

	Variegated red		*Variegated white*		*Red*		*White*	
	♀	♂	♀	♂	♀	♂	♀	♂
Observed	76	35	0	34	27	5	0	6
Expectation	*74*	*37*	*0*	*34*	*21.4*	*10.7*	*0*	*6*

DISCUSSION

Color-producing genes

White is recessive to any other color and breeds true (experiment 13). Red and white, when crossed, segregate out, each to its respective original color in the F_2 generation, according to the simplest Mendelian ratio (experiments 7, 8, 19, 20), from which we see that red color is produced by a single dominant gene which I will call *R*. The cross between brown and red gives also in F_2 the original colors according to the 3:1 ratio (experiment 1), from which we see clearly that brown color has one more gene, *B*, in addition to the red-producing *R*. That the genetical constitution of brown should be *BR* is confirmed by the cross of brown with white, because in the F_2 generation four varieties, brown, blue, red, and white, are produced according to the dihybrid ratio, 9:3:3:1 (experiments 10, 17). The same fact is more directly proven by the result of experiment 12 where the cross blue × red gave rise to brown offspring. Blue individuals, which originated in my cultures, possess the gene *B*, which is also contained in brown ones, as shown by the results of experiments 14, 15 and 16. TOYAMA (1916) and ISHIWARA (1917) have reported results exactly similar to mine derived from crosses between brown, red, and white.

Variegation is produced by another gene, *B'*; this is clearly proven by the cross between variegated red and red (experiments 5, 6), where we see the monohybrid segregation, as well as by the cross between variegated red and white (experiments 9, 21) where we see the dihybrid segregation concerning the genes *B'* and *R*.

The action of the above-mentioned color-producing genes may be regarded as follows:

B is a gene able to produce black pigment or melanophore uniformly throughout the whole body;

R is a gene able to produce yellow pigment or xanthophore;

B' is a gene able to produce black pigment only partially in the body.

In accordance with the foregoing hypotheses, the genetic constitution of the several color varieties, when homozygous, would be: brown, *BBRR;* blue, *BBrr;* red, *bbRR;* variegated red, *B'B'RR;* variegated white, *B'B'rr;* and white, *bbrr.*

Triple allelomorphs

The three colors, brown, variegated red, and red, form a triple allelomorphic series. Any two of them, when crossed, show in F_2 the 3:1 segregation (experiments 1, 3, 5). Brown is dominant to variegated red as well as to red, while variegated red is dominant to red. When brown and red are crossed, the F_2 offspring produce brown and red in the ratio 3:1, and do not contain any variegated red, which is the characteristic behavior of multiple allelomorphs.

A similar mode of inheritance was described by IBSEN (1919) in respect to the black, tortoise, and red colors in guinea-pigs, which are produced, respectively, similarly as in our three varieties, by complete extension, partial extension, and non-extension of black pigment.

Sex-linked inheritance

The results of experiments 7 to 11 as well as 17 to 21 attract our attention on account of a remarkable manifestation of sex-linked inheritance. When a white female (*rr*) is mated to a male possessing the *R* gene in a homozygous state, all F_2 white offspring (*rr*) as a rule are female. Thus, in experiment 8 all white individuals except one, were female, and in the cross, white female × variegated red male (experiment 9), all white and variegated white individuals were female, and in the cross white female × brown male (experiments 10, 11), all blue and all white individuals, except one of each, were female.[1]

On the contrary, when a white male is mated to a female possessing the *R* gene in a homozygous state, all F_2 white, blue or variegated white individuals as a rule are male (experiments 17, 19, 21).

All these peculiar results may be explained in the following way: First, the sex inheritance of the fish under discussion belongs to the Drosoph-

[1] That all whites are female without exception has been noticed by TOYAMA (1916), but ISHIWARA (1917) had both male and female whites in his experiments.

ila type, XX–XY, i.e., to that type in which the female is homozygous and the male heterozygous for the sex (or X) chromosome. HUXLEY (1920, page 270) recently assumed the same type of sex inheritance to occur in the fish, Giardinus. Second, the red-producing gene R is located in the chromosomes X and Y.

The male homozygous for red color has one R gene contained in the X and another contained in the Y chromosome, which I represent by R_x and R_Y, respectively; while the female homozygous for red color has one R gene contained in each of the two X chromosomes which I represent by R_x.

As a critical test for the above view the results of experiment 8 may be cited. In this experiment the male F$_1$ offspring derived from the cross white female × homozygous red male were mated to white females. Had the sex been inherited according to the Abraxas type, in which the female is heterozygous and the male homozygous in respect to the sex-determining genes, we should expect to have as the result of the experiment, red females and males, and white females and males in equal numbers. The actual result was quite different; all whites, 197 in number, were female except only one individual, while all reds, except 2 out of 253, were male. When we assume the Drosophila type in our case, these results are in perfect agreement with the theoretical expectation, as we shall see in the following diagram.

$r_X r_X$ (white ♀) × $R_X R_Y$ (red ♂)

$R_X r_X$ (red ♀) $\qquad$ $r_X R_Y$ (red ♂) × $r_X r_X$ (white ♀)

$r_X r_X$ (white ♀) (all white) $\qquad$ $r_X R_Y$ (red ♂) (all red)

As above stated, the mode of sex inheritance in our fish belongs to the Drosophila type. The Y chromosome in our case differs however from what we see in Drosophila, inasmuch as it contains the dominant gene R. The production of *red* (*dominant*) males from the cross between *white* (*recessive*) female and *red* (*dominant*) male, as seen in the above diagram, would be otherwise quite unexplainable.

All other experimental results concerning sex linkage are equally well explainable according to the above hypothesis. Below I will give in the form of diagrams the theoretical expectation and the results actually obtained in experiments 7, 9, 10, 11, 17, 18, 19, 20, and 21.

Experiment 7

R_XR_Y (red ♂) × r_Xr_X (white ♀)

R_Xr_X (red ♀) × r_XR_Y (red ♂)

R_Xr_X (red ♀) r_Xr_X (white ♀) R_XR_Y (red ♂) r_XR_Y (red ♂)

	Red		White	
	♀	♂	♀	♂
Theoretical sex ratio	1	2	1	0
Observed	41	76	43	0

Experiment 9

$B'B'R_XR_Y$ (variegated red ♂) × bbr_Xr_X (white ♀)

$B'bR_Xr_X$ (variegated red ♀) × $B'br_XR_Y$ (variegated red ♂)

$\left.\begin{array}{r} B'B'R_XR_Y \\ B'B'r_XR_Y \\ 2B'bR_XR_Y \\ 2B'br_XR_Y \end{array}\right\}$ (variegated red ♂)

$\left.\begin{array}{r} B'B'R_Xr_X \\ 2B'bR_Xr_X \end{array}\right\}$ (variegated red ♀)

$\left.\begin{array}{r} B'B'r_Xr_X \\ 2B'br_Xr_X \end{array}\right\}$ (variegated white ♀)

bbR_Xr_X (red ♀)

$\left.\begin{array}{r} bbr_XR_Y \\ bbR_XR_Y \end{array}\right\}$ (red ♂)

bbr_Xr_X (white ♀)

	Variegated red		Variegated white		Red		White	
	♀	♂	♀	♂	♀	♂	♀	♂
Theoretical sex ratio....	1	2	1	0	1	2	1	0
Observed............	46	90	59	0	17	37	17	0

Experiment 10

BBR_XR_Y (brown ♂) × bbr_Xr_X (white ♀)

BbR_Xr_X (brown ♀) × Bbr_XR_Y (brown ♂)

$\left.\begin{array}{r} BBR_XR_Y \\ BBr_XR_Y \\ 2BbR_XR_Y \\ 2Bbr_XR_Y \end{array}\right\}$ (brown ♂)

$\left.\begin{array}{r} BBR_Xr_X \\ 2BbR_Xr_X \end{array}\right\}$ (brown ♀)

$\left.\begin{array}{r} BBr_Xr_X \\ 2Bbr_Xr_X \end{array}\right\}$ (blue ♀)

bbR_Xr_X (red ♀)

$\left.\begin{array}{r} bbR_XR_Y \\ bbr_XR_Y \end{array}\right\}$ (red ♂)

bbr_Xr_X (white ♀)

	Brown ♀	Brown ♂	Blue ♀	Blue ♂	Red ♀	Red ♂	White ♀	White ♂
Theoretical sex ratio.....	1	2	1	0	1	2	1	0
Observed*..............	148	211	115	0	57	168	81	0

*Sum of the results of two experiments, 10 A and 10 B.

Experiment 11

Bbr_XR_Y (brown♂) × bbr_Xr_X (white♀)

Bbr_XR_Y (brown♂) Bbr_Xr_X (blue♀) bbr_XR_Y (red♂) bbr_Xr_X (white)

	Brown ♀	Brown ♂	Blue ♀	Blue ♂	Red ♀	Red ♂	White ♀	White ♂
Theoretical sex ratio.....	all ♂		all ♀		all ♂		all ♀	
Observed (the sum of the results of two experiments, 11 A and 11 B)	2	286	328	1	0	206	208	0

Experiment 17

BBR_XR_X (brown♀) × bbr_Xr_Y (white♂)

BbR_Xr_X (brown♀) × BbR_Xr_Y (brown♂)

$\left.\begin{array}{l} BBR_XR_X \\ BBR_Xr_X \\ 2BbR_XR_X \\ 2BbR_Xr_X \end{array}\right\}$ (brown♀)

$\left.\begin{array}{l} BBR_Xr_Y \\ 2BbR_Xr_Y \end{array}\right\}$ (brown♂)

$\left.\begin{array}{l} BBr_Xr_Y \\ 2Bbr_Xr_Y \end{array}\right\}$ (blue♂)

$\left.\begin{array}{l} bbR_XR_X \\ bbR_Xr_X \end{array}\right\}$ (red♀)

bbR_Xr_Y (red♂)

bbr_Xr_Y (white♂)

	Brown ♀	Brown ♂	Blue ♀	Blue ♂	Red ♀	Red ♂	White ♀	White ♂
Theoretical sex ratio.....	2	1	0	1	2	1	0	1
Observed..............	173	88	0	79	78	34	0	31

Experiment 18

BbR_Xr_Y (brown♂) × bbr_Xr_X (white♀)

BbR_Xr_X (brown♀) Bbr_Xr_Y (blue♂) bbR_Xr_X (red♀) bbr_Xr_Y (white♂)

	Brown ♀	Brown ♂	Blue ♀	Blue ♂	Red ♀	Red ♂	White ♀	White ♂
Theoretical sex ratio.....	all ♀		all♂		all♀		all♂	
Observed..............	157	0	0	148	165	0	0	126

Experiment 19

R_XR_X (red ♀) × r_Xr_Y (white ♂)

R_Xr_X (red ♀) × R_Xr_Y (red ♂)

R_XR_X (red ♀) R_Xr_X (red ♀) R_Xr_Y (red ♂) r_Xr_Y (white ♂)

	Red ♀	Red ♂	White ♀	White ♂
Theoretical sex ratio	2	1	0	1
Observed	87	42	0	33

Experiment 20

R_Xr_Y (red ♂) × r_Xr_X (white ♀)

R_Xr_X (red ♀) r_Xr_Y (white ♂)

	Red ♀	Red ♂	White ♀	White ♂
Theoretical sex ratio	all ♀		all ♂	
Observed	135	0	0	153

Experiment 21

$B'B'R_XR_X$ (variegated red ♀) × bbr_Xr_Y (white ♂)

$B'bR_Xr_X$ (variegated red ♀) × $B'bR_Xr_Y$ (variegated red ♂)

$B'B'R_XR_X$, $B'B'R_Xr_X$, $2B'bR_XR_X$, $2B'bR_Xr_X$ (variegated red ♀)

$B'B'R_Xr_Y$, $2B'bR_Xr_Y$ (variegated red ♂)

$B'B'r_Xr_Y$, $2B'B'r_Xr_Y$ (variegated white ♂)

bbR_XR_X, bbR_Xr_X (red ♀)

bbR_Xr_Y (red ♂)

bbr_Xr_Y (white ♂)

	Variegated red ♀	Variegated red ♂	Variegated White ♀	Variegated White ♂	Red ♀	Red ♂	White ♀	White ♂
Theoretical sex ratio	2	1	0	1	2	1	0	1
Observed	76	35	0	34	25	5	0	6

Location of the dominant gene R in the Y chromosome

One of the notable features in the sex-linked inheritance above described is the fact that the Y chromosome contains the dominant gene R. All red males bought from the fancier were homozygous for that color. That the wild brown male contains the R gene in its Y chromosome is to be seen clearly from the results of experiments 10 and 11. Since the Y chromosome is inherited by the male, exclusively, and consequently goes from the father to his son only, all males contained in the progeny of any mating to which either a red or a brown male is a party, possess the gene R, i.e., they are red, red variegated, or brown; whilst all individuals lacking the gene R, i.e., white, white variegated, or blue, are female (experiments 7, 8, 9, 10 and 11).

According to the investigations hitherto reported, the Y chromosome in the Drosophila type and also the W chromosome in the Abraxas type (corresponding to the Y chromosome in Drosophila) are known to possess no dominant gene, so that all characters contained therein are recessive in genetic behavior. The fact that in our fish this chromosome carries a dominant gene instead of a recessive is very interesting, and will bring out a certain important significance of its existence.

Crossing over between the sex chromosomes

No example of crossing over between a sex chromosome and any other chromosome has been reported till now, and it has been supposed that, because this is so in the heterozygous sex, the X and Y chromosomes are not exactly homologous to each other so as to permit the crossing over of genes between them. In my experiments the crossing over of the R gene between them seems not to be very rare. In experiment 8, in which all red were expected to be male and all white to be female, two reds out of 253 were female ($R_X r_X$) and one white out of 198 was male ($r_X r_Y$). In experiment 11 A, where all blues were expected to be female, one out of 215 was male ($Bbr_X r_Y$); and in experiment 11 B, where all browns were expected to be male, two out of 122 were female ($BbR_X r_X$).

The production of such unexpected individuals is, in my opinion, best explained by assuming the crossing over of the R gene from a Y to an X chromosome. One might perhaps think that it may be caused by the process of non-disjunction discovered by BRIDGES (1916). But if this were the correct explanation, all exceptional females would have three sex chromosomes, viz., two X and one Y chromosome, and all exceptional males would have only one X chromosome. Thus the constitution of the red female in experiment 8 should be $r_X r_X R_Y$, that of the brown female in experiment 11 B should be $Bbr_X r_X R_Y$, that of the white male in experiment 8 should be $r_X 0$, and finally that of the blue male in experiment 11 A should be $Bbr_X 0$. The results of experiments 17, 18, 19, 20, and 21, in which the male offspring of the exceptional white male derived from experiment 8 were mated to various females, would then have to be explained by assuming the constitution of the original male to have been r_X0.

In order to settle the question, which of the two views,—crossing over or non-disjunction,—will better explain the production of unexpected individuals, the result may be cited of a mating between heterozygous red females and males (experiment 22, not previously described in this paper).

The red mothers in this experiment were F_2 offspring from the cross white ♀ × brown ♂ (experiment 10 B) and should have the constitution $R_X\ r_X$, while the red fathers were F_2 offspring from the cross brown ♀ × white ♂ (experiment 17) and should have the constitution either R_Xr_X or R_X0, according as we adopt the view of crossing over or that of non-disjunction. The result of the cross was

Experiment 22 (1919)

Red ♀ (R_Xr_X) × Red ♂ (R_Xr_Y or R_X0)

	Red	White
Observed	451	149
Expectation	*450*	*150*

The result of sex examination in 1920 was

	Red		*White*	
	♀	♂	♀	♂
Observed	146	57	2	80
Expectation	*135.4*	*67.7*	*0*	*82*

Here also two unexpected white females were produced. If we consider the constitution of the red male to be R_X0, the production of the white females is not explainable, because the white female should have two X chromosomes, i.e., should contain r_Xr_X, but in the cross of R_Xr_X female with R_X0 male there would be but one single gene r. By assuming the constitution of the red male to be R_Xr_Y and supposing moreover that the crossing over takes place between the chromosomes R_X and r_Y, so that chromosomes r_X and R_Y are thereby formed, the production of r_Xr_X may clearly be explained. We are led consequently to the conclusion that the production of the exceptional red females and the white male in experiment 8 is due, not to the process of non-disjunction, but to the crossing over of the R gene between the X and the Y chromosomes. As to exceptional individuals produced in experiment 11 my endeavors to determine their origin have been thus far in vain, though their production by crossing over seems to me to be highly probable. At present, my data concerning this question are yet very scanty, and I am unable to make any further inference in this respect, but I hope to be able to give much more detail in a future paper, because the experiments for the purpose are now in progress.

SUMMARY

1. In *Aplocheilus latipes* Temmick and Schlegel, the wild form is brownish black but four novel color varieties are known, viz., orange-red, orange-red variegated with black, white, and white variegated with black.

2. The genetic consequences of a mating between any of the three forms, brown, red, and white, are quite simple. Brown is produced by the genes B and R, red by the gene R and white contains no dominant gene.

3. In the F_2 generation of the cross between brown and white a new variety of a blue-black color first made its appearance. It is characterized by the dominant gene B.

4. Variegation is produced by the gene B'. The three genes, B, B', and the recessive b, form a series of triple allelomorphs.

5. Sex inheritance is of the Drosophila type, XX–XY, i.e., the female is homozygous and the male heterozygous in respect to the sex-determining genes.

6. In the male homozygous for the R gene, one R is carried by the Y chromosome. Since this chromosome is transmitted from male to male, whatever the genetical constitution of the female may be, all male progeny contain the R gene, and consequently neither white nor blue males are normally produced.

7. Crossing over of the R gene between the X and the Y chromosomes was found not rarely in the offspring of back-crosses between heterozygous brown or red males and white females and also matings of heterozygous red males with heterozygous red females.

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POSTSCRIPT.—The manuscript of the present paper was sent to Dr. SHULL at the end of April 1921. On the last day of May, "Science," Vol. 53, No. 1371, published April 8, came to my hands, and on reading there the paper of Dr. CASTLE, entitled "A new type of inheritance" (pp. 339–342), I first became acquainted with the investigation of Dr. SCHMIDT of Copenhagen, concerning the "Millions fish," *Lebistes reticulatus*. Though I am not yet able to get his original paper, published in "Comptes rendus des Travaux du Laboratoire de Carlsberg," Vol. 14, No. 8, 1920, his observations as described in "Science" are as follows: A conspicuous black spot occurring on the dorsal fin of the male in one race of this species is transmitted exclusively from father to son, regardless of the mother's ancestry. Several cross-experiments have led SCHMIDT to the conclusion that the dominant gene causing that sex-linked character must be located in the Y chromosome, which has been considered till now to be quite "empty," especially in Drosophila.

The discovery of Dr. SCHMIDT consequently accords well with what I have observed in *Aplocheilus latipes*, and have described in the present paper, though the two cases differ in several minor details.[2] It is very interesting that the new mode of inheritance of exactly similar type was found by Dr. SCHMIDT and me quite independently from each other at almost the same time, especially so because both relate to the same animal group—the fishes.

[2] EDITORIAL NOTE. It should be pointed out, in justice to the author, that his observations go beyond those of SCHMIDT in the important respect of showing the occurrence of crossing over between the X and the Y chromosomes.—W. E. CASTLE.